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A survey of antibiotic residues in commercial eggs in Kermanshah, Iran

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Abstract

Poultry nutrition or inappropriate treatments by antibiotics can lead to production of eggs with antibiotic residues. The aim of the present study is to assess the prevalence of drug residues in eggs presented in Kermanshah, Iran. For this purpose, 120 eggs with the average weight of 60 grams were collected randomly from the supermarkets in Kermanshah and later were tested for antibiotic residues using the Four- Plate Test (FPT) method. The results showed that 3.3% of the tested eggs had antibiotic residues. The main residues in egg yolk was due to tetracycline. Aminoglycosides were also detected as minor contaminants. Accordingly, antibiotic residues in eggs presented in Kermanshah are a threat for human consumption and therefore strong supervision of the authorities is essential.

Keywords: Antibiotic residues, Egg, FPT method, Kermanshah.

Introduction

Antibiotics are groups of chemical compounds produced biologically by some plants and microorganisms like fungus and have antibacterial or growth preventing effects on bacteria, and are considered as the final strategy to treat infections in human beings. In addition to their beneficial effects, inappropriate use of antibiotics can generate the most risks to humans (Smith *et al.*, 2009). Heretofore, more than 40,000 antibiotics have been recognized and 80 of them are being used in agriculture and aquaculture (Martos *et al.*, 2010). Antibiotics are used on a large scale in poultry farms to cure or to prevent diseases and also to promote growth which leads to an increase in poultry production and a more reasonable price of poultry products; On the other hand, high concentration of antibiotics in poultry tissues is a threat for the consumers. Poultry would store drug residues in egg yolks for a few days or even weeks after the dosing period (Donoghue *et al.*, 1996). Although some of these antibiotics are approved in poultry industry, extensive monitoring is still needed to ensure the safety of foods. International organizations like WHO, FAO, EU and FDA have determined maximum tolerance levels or *acceptable daily intake a*nd withdrawal times of active pharmaceutical ingredients for poultry before consumption (Al-Ghamdi *et al.*, 2000).

Over the past few years, several diagnostic methods have been developed for detecting antibiotic residues in foods and edible tissues of animals such as microbiological methods, immunochemical methods and quantitative measurements of drug residues by chromatography methods. Each one of these methods has its own advantages and disadvantages. Among them, microbiological methods are the most common and practical methods for screening of antibiotic residues in foods, due to their economic advantages, compatibility to be performed in large scales and their detection of multiple types of antibiotics (Ehsani & Hashemi, 2015; Fabiansson & Rutegård, 1978). Four-Plate Test method is a favorable microbiological screening method. It is based on the formation of an inhibition zone around the samples in four culture media with different pH (6, 7.2, 8) and different bacteria (Ehsani & Hashemi, 2015). Therefore, in this study the presence of antibiotic residues was investigated by the FPT method in eggs distributed in Kermanshah, a city in west of Iran.

Materials and methods

Sample preparation

A total of 120 commercial eggs with the average weight of 60 grams were collected from retail markets in various parts of Kermanshah. The egg samples were then taken to the laboratory for analysis.

Preparation of culture media

FPT was performed by using two culture media: Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) and Mueller Hinton Agar (MHA, Merck, Darmstadt, Germany). *Bacillus subtilis (*ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) were prepared and taken from Iranian Research Organization for Science and Technology and were activated according to the instructions. The bacteria were transferred to TSB and incubated at 37°C to achieve necessary turbidity. The bacterial suspensions were adjusted by a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden) at 600 nm to achieve OD: 0.085-0.1, a concentration approximately equal to 0.5 McFarland turbidity (1.5×10^8 CFU.mL^{-1}) and inoculated by sterile cotton swab sticks on agar media. Agar media for *Bacillus subtilis* were prepared in three different pH levels (6, 7.2 and 8) and for *staphylococcus aureus* were adjusted to pH 8, using hydrochloric acid and sodium hydroxide and autoclaved. The bacteria were *B. subtilis*, 1.0×10^5 CFU ml^{-1} and *S. aureus*, 1.5×10^6 CFU ml^{-1}.

Testing of samples

Each egg surface was cleaned using a sterile hand towel soaked in 70% (v/v) alcohol. The albumen was drained out of a small pore made on the egg surface using sterile forceps. Egg yolks were then transferred to sterile falcon tubes. The homogenization step was performed by adding 10 ml of phosphate buffer (pH 7) to the egg yolk. Special sterile paper discs, 12 mm in diameter, were then dipped into the falcon containing egg yolk. Finally discs were placed onto the surface of inoculated MHA (Ehsani & Hashemi, 2015; Kabir *et al.*, 2004).

After incubation at 37°C for 24 h, samples with inhibition zones were considered as positive and their diameters were measured. Five different antibiotic groups were considered: β-lactams, tetracyclines, sulfonamides, aminoglycosides and macrolides (G. Okerman *et al.,* 2000). Detecting the ability of bacteria according to the type of culture media is summarized in Table 1. Tetracycline (30 µg), trimethoprim (25 µg), gentamicin (10 µg) and erythromycin (15 µg) discs were used as control positive standards.

Results

Inhibition zone of about 2mm around a disc was considered as a positive result. The results of 120 tested samples revealed that 4 samples were positive (3.3%). Three samples (2.5%) were positive at pH 6, showing penicillin and tetracycline residues and 1 sample (0.8%) was positive at pH 8 of *Bacillus subtilis*, showing aminoglycoside residues (Table 1). Inhibition zone of control positive discs are summarized in Table 2.

Table1. Antibiotic detection by the FPT method

pH of culture medium	Tested bacteria	Detected antibiotic	NO of positive samples
6	*Bacillus subtilis*	Penicillin&Tetracycline groups	3(2.5%)
7.2	*Bacillus subtilis*	Sulfonamide group	0
8	*Bacillus subtilis*	Aminoglycoside group	1(0.8%)
8	*Staphylococcus aureus*	Penicillin&Macrolide groups	0

Table 2. Diameter of inhibition zone (mm) of control positive discs

Antibiotic	pH 6 B	pH 7.2 B	pH 8 B	pH 8 S
Tetracycline	37	25	20	10
Trimethoprim	36	36	40	26
Gentamycin	22	26	35	30
Erythromycin	21	21	25	32

B: Bacillus subtilis
S: Staphylococcus aureus

Discussion

Antibiotics, at subtherapeutic levels, are used in food animals such as poultry for growth promotion, weight gain, higher efficiency and/or prevention of one or more diseases (Vazquez-Moreno *et al.*, 1990). Feeding animals with foods containing antibiotics is harmful for human health, causing allergy and antibiotic resistance (Ansari & Khatoon, 1994). Screening is the first step to prove drug residues. These methods should be inexpensive, applicable for multiple samples and should be able to show the lowest false negative and false positive results (Mariel, 2008). Qualitative methods such as microbiological methods use antibiotic residues screening in foods especially foods with animal origin (Okerman e*t al.*, 1998). FPT is a reference method for foods screening and detecting the main category of antibiotic residues in the EU (Kilinc & Cakli, 2008).

According to the results of this study, the

highest contamination rate of antibiotic residues was related to penicillin and tetracycline groups because of their physicochemical and pharmacokinetic properties and physiological condition of poultry (Donoghue *et al.*, 1996; Furusawa, 1999). Hakimzadegan *et al*. (2014) investigated the presence of antibiotic residues in egg yolk using the FPT method and reported that 16.66% of eggs were contaminated with Tetracycline but major contaminants in their study were Macrolides (61.11%) (Hakimzadegan *et al.*, 2014). Ehsani and Hashemi (2015) reported that twenty five samples (12.5%) of the eggs in Urmia, a city in northwest of Iran, were positive for antibacterial substances which were related to Macrolides group.

In some other studies on egg yolk using the FPT method, Macrolides were also the major detected antibiotics (Smith *et al*., 2007) and aminoglycosides and tetracycline were detected as minor contaminants. Different results in former studies can be due to the various antibiotics used to cure poultry diseases in different geographical regions (Kabir *et al.,* 2004). Chowdhury e*t al*. (2015) reported tetracycline, amoxicillin, and ciprofloxacin residues in commercial eggs through microbial inhibition test and thin layer chromatography. Tetracyclines are approved for treating poultry with a maximum limit of 400 µg/kg (CAC, 2012) and the results of the present investigation indicate that there is a widespread misuse of tetracycline in egg layer chicken farms in Kermanshah due to their broad spectrum against gram positive and gram negative bacteria.

Since it is more appropriate to screen the egg yolk than the egg white for antibiotic residues by the FPT method due to the presence of lysozyme in the egg yolk (Alm El Dein and Elhearon, 2010), in this study only antibiotic concentration of the egg yolk was investigated.

In conclusion this study indicates that a broader control of antibiotic administration in food animals as well as a stricter monitoring of breeders along with a substantial supervision on drug withdrawal time should be conducted in this region of Iran. Moreover, preventive programs such as vaccination should be developed in order to reduce antibiotic administration in poultry industries and to decrease the drug residues in food animal.

Conflicts of interest

The authors declare no conflict of interest.

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بررسی باقیمانده های آنتی بیوتیکی در تخم مرغ های تجاری شهر کرمانشاه، ایران

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چکیده

تغذیه یا درمان نامناسب طیور توسط آنتی بیوتیک ها موجب تولید تخم مرغ هایی با باقی مانده های آنتی بیوتیکی می گردد. هدف از بررسی حاضر ارزیابی باقیمانده های دارویی در تخم مرغ های عرضه شده در شهر کرمانشاه، ایران می باشد. به این منظور 120 تخم مرغ با میانگین وزنی 60 گرم بصورت تصادفی از فروشگاه های کرمانشاه جمع آوری شد و در مرحله بعدی با روش آزمون چهار پلیتی، باقیمانده آنتی بیوتیکی اندازه گیری شد. نتایج نشان داد که 3,3% تخم مرغ های بررسی شده دارای باقیمانده آنتی بیوتیکی بودند. اصلی ترین باقیمانده در زرده، تتراسایکلین بود. آمینوگلیکوزید ها نیز به عنوان آلوده کننده های جزئی شناسایی شدند. بر این اساس باقیمانده آنتی بیوتیکی در کرمانشاه تهدیدی برای مصرف انسانی بوده و بنابراین نظارت قوی مسئولین ضروری به نظر می رسد.

واژگان کلیدی: باقیمانده های آنتی بیوتیکی، تخم مرغ، روش آزمون چهار پلیتی، کرمانشاه